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# Molecular Biology and Information

## - Molecular Biology -

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## Scope of Research

The major subjects are mechanisms involved in signal transduction and regulation of gene expression responsive to environmental stimuli, differentiation and development of plant organs, and plant-microbe interaction. As of December 2002, study is being concentrated on the roles of two-component response regulators and homeodomain proteins of higher plants in signal transduction and developmental processes.

## Research Activities (Year 2002)

### Presentations

The transcription factor-type response regulator ARR1 is involved in an early step of cytokinin signal transduction, Oka A, Aoyama T, Honma T, Sakai H, 2002 Ann Meeting of Jpn. Soc. Plant Physiol.: Symposium 'Phytohormone signal transduction for physiological functions', 28 - 30 March (Okayama).

Transcription factors ARR1 and ARR2 controlling cytokinin responses, Aoyama T, RIKEN Symposium 'Signal perception and transduction in higher plants', 26 April (Wako).

Isolation and analysis of direct target genes for the transcriptional regulation of ATHB-1, Muramoto T, Oka A, Tabata S, Lucchetti S, Morelli G, Ruberti I, Aoyama T; Initial step of root hair morphogenesis regulated by the transcription factor GLABRA2, Ohashi Y, Oka A, Pousada RR, Ruberti I, Morelli G, Aoyama T, 28 June - 2 July, XIII International Conference on Arabidopsis Research (Seville, Spain).

### Grants

Oka A, Research project for network mutually controlling plant responses to environmental stimuli with morphogenesis: Hierarchy of transcriptional controls in plant signal transduction, Special Coordination Fund of the Ministry of Education, Culture, Sports, Science, and Technology of Japan, 1 April 1997 - 31 March 2003.

Aoyama T, Functional analysis of homeodomain proteins controlling the flexibility of plant morphogenesis, Grant from the Bio-oriented Technology Research Advancement Institution (BRAIN), 1 April 1998 - 31 March 2003.

Aoyama T and Oka A, Molecular mechanism of adaptive responses controlled by *Arabidopsis* His-Asp phosphorelay signal transduction, Grant-in-Aid for Scientific Research on Priority Areas (B), 1 April 2000 - 31 March 2005.

Sakai H, Molecular basis of cytokinin signalling in plant cells, Grant-in-Aid for Scientific Research on Priority Areas (A), 1 April 2002 - 31 March 2003.

## The *Arabidopsis* response regulator ARR1 is a transcription factor for genes immediately responsive to cytokinins

Bacteria have devised phosphotransfer signalling mechanisms for eliciting a variety of adaptive responses to their environment. These mechanisms are collectively referred to as two-component regulatory systems. Each system generally consists of a sensor protein histidine kinase, which is anchored in the cell membrane, and a cytoplasmic response regulator, whose activity is modulated by the sensor. Most response regulators are transcription factors. We have recently presented the evidence for the existence of quite similar systems in higher plants, such as the signal transduction induced by the phytohormone cytokinin [1, 2]. The *Arabidopsis* CRE1 histidine kinase and its related proteins *AHK2* and *AHK3* perceive cytokinins in the environment and transduce a signal, presumably through the AHP bridge components that carry the histidine-containing phosphotransfer (HPT) domain, to the ARR1 response regulator that transcriptionally activates genes (e.g. *ARR6*) immediately responsive to cytokinins (Figure 1).

The *Arabidopsis* genome additionally codes for 10 ARR1-like response regulators, most of which also appear to be involved in cytokinin signalling. Besides, the *Arabidopsis* genome provides another type of 11 response regulators (e.g. *ARR6*) that are not transcription factors. They are quickly induced by cytokinins through ARR1 directly. Although ethylene and daylight signalling are also triggered by sensor histidine kinases (e.g. *ETR1* and *PHYB*), no corresponding response regulators have been identified. Therefore, the cytokinin signalling process may participate in cross-talk with signalling systems that respond to ethylene and daylight, through an intra-

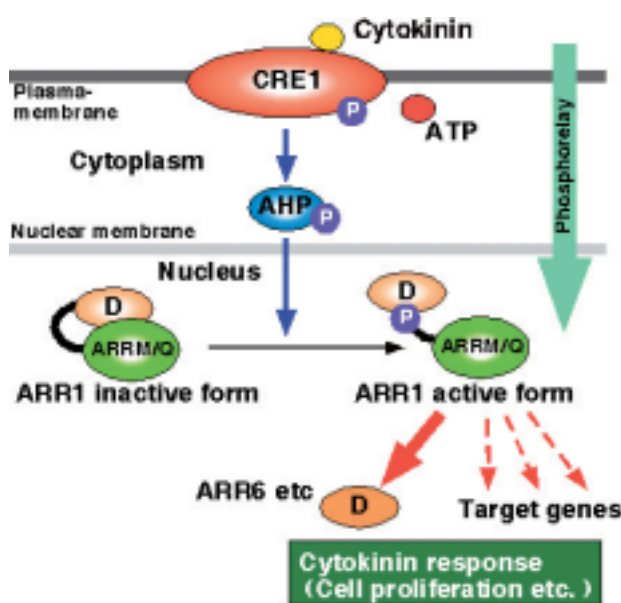


Figure 1. Model of cytokinin signal transduction.

cellular pool of several ARR1-like molecular species.

1. H. Sakai, T. Aoyama, and A. Oka, *Plant J.*, **24**, 703-711 (2000).
2. H. Sakai, T. Honma, T. Aoyama, S. Sato, T. Kato, S. Tabata, and A. Oka, *Science*, **294**, 1519-1521 (2001).

## Entopically additive expression of *GLABRA2* alters the frequency and spacing of trichome initiation

During plant development, proliferated cells undergo morphological and physiological differentiation that allows specialized function. Among various cell types in plants, *Arabidopsis* trichomes have been used as a model to study plant cell differentiation. A trichome is a hair-like structure on the surface of a plant shoot, consisting of a single cell that develops in a series of cellular events, including endoreduplication, cell expansion and outgrowth, branching, and cell wall maturation. As trichomes are not essential for growth, many mutants defective in trichome development have been isolated. Most of these mutants are divided into two classes based on their phenotypes. The first class participates in the initiation of trichome development, e.g. *GLABRA1* (*GL1*) and *TRANSPARENT TESTA GLABRA1* (*TTG1*). Defects of this class change the initiation frequency or spacing of trichomes. The second class is related to trichome cell morphogenesis. Defects in this class result in the generation of aberrant trichomes.

*GLABRA2* (*GL2*)/*ATHB-10* categorized in the second class encodes a homeodomain protein that belongs to the homeodomain-leucine zipper family. *GL2* is involved in not only trichome development, but also root hair and seedcoat development. We have here studied the role of *GL2* in trichome development [3]. A transgene consisting of a *GL2*-coding fragment preceded by the cauliflower mosaic virus 35S promoter (*35S::GL2*) does not complement *gl2-1* mutant defects. In the wild-type genetic background, *35S::GL2* causes *gl2*-mutant-like and scarcely viable phenotypes, suggesting that ectopic overexpression of *GL2* interrupts endogenous *GL2* function in trichome development, being toxic to plants. On the other hand, another *GL2* transgene containing the *GL2* promoter (*pGL2::GL2*) complements the *gl2-1* defects. Entopically additive expression of *GL2* by introduction of *pGL2::GL2* in the wild-type genetic background noticeable increases the number of trichomes and induces production of adjacent trichomes. In addition, *gl2-1/+* heterozygous leaves have fewer trichomes than *+/+* wild-type leaves. These results indicate that the amount of *GL2* in cells correlate with the frequency of trichome initiation, thereby being involved in determining trichome spacing.

3. Y. Ohashi, A. Oka, I. Ruberti, G. Morelli, and T. Aoyama, *Plant J.*, **29**, 359-369 (2002).